

# **Axl and MerTK regulate synovial inflammation and are modulated by IL-6 inhibition in rheumatoid arthritis**

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## **AFFILIATIONS**

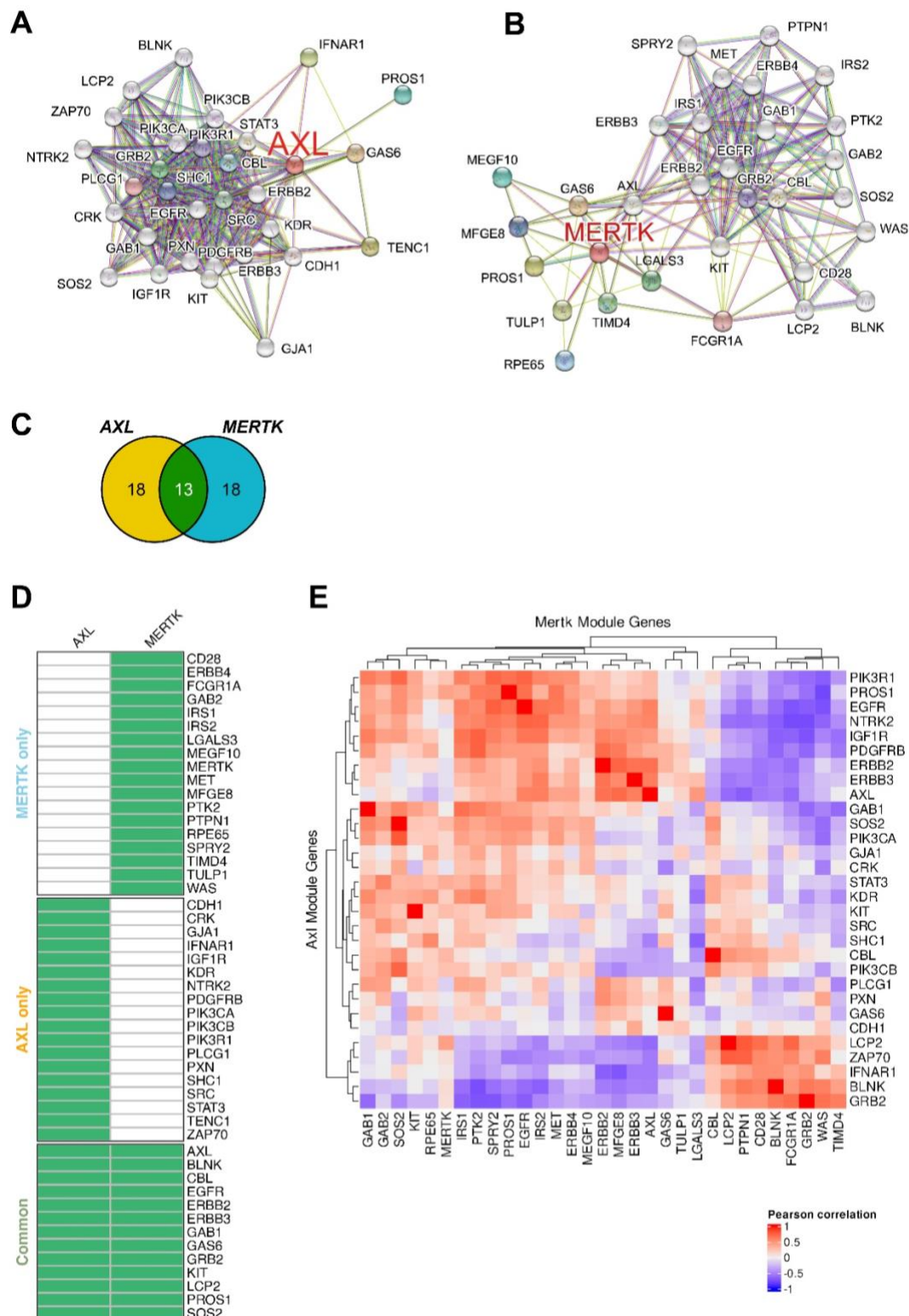
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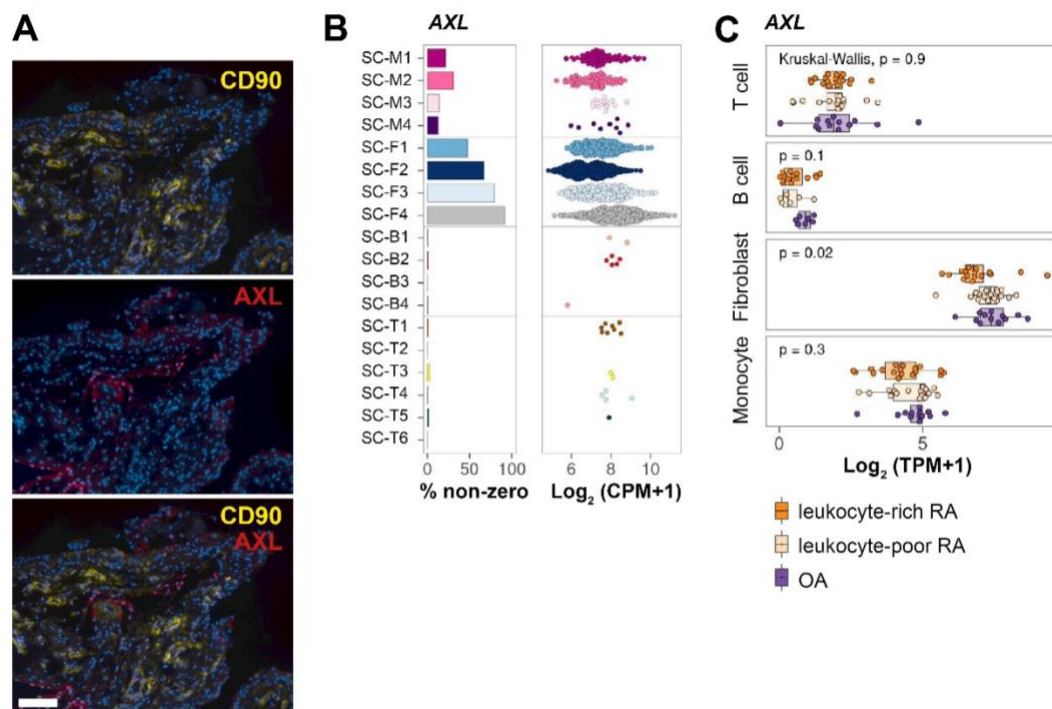
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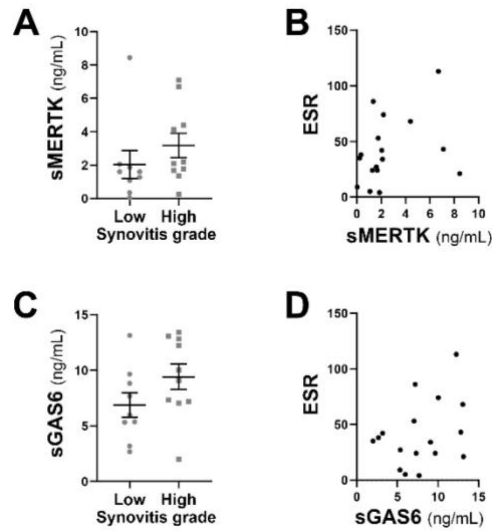
**Supplementary Figure 1 AXL and MERTK STRING networks partially overlap.**

(A, B) STRING functional AXL (A) and MERTK (C) protein network (<https://string-db.org/>). (C) Venn's diagram showing overlapping versus AXL- and MERTK-specific partner genes. (D) Detailed list of genes in the AXL and MERTK networks. (E) Heatmap showing the Pearson correlation coefficient between all genes in the AXL and MERTK modules.



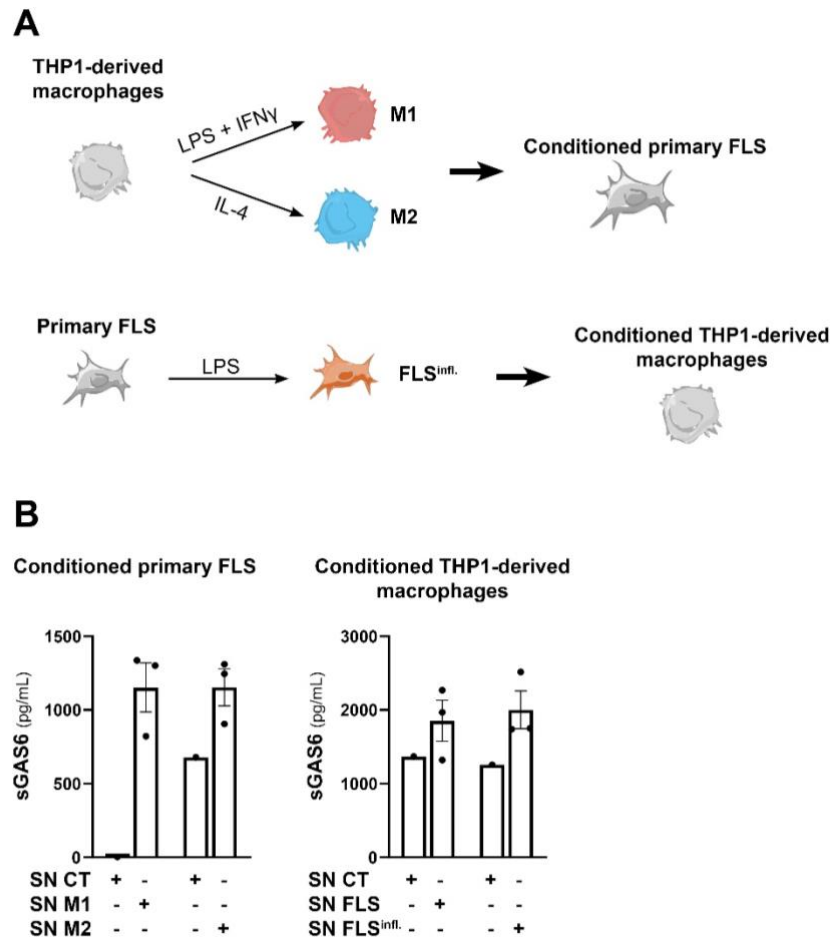
**Supplementary Figure 2** Axl expression in fibroblasts and scRNA-seq/bulk RNA-seq expression in public databases.

**(A)** Double immunostaining of CD90 (yellow) and Axl (red) in the synovium of RA patients showing no co-localization between both proteins. Nuclei were counterstained with DAPI (blue). Scale bar = 50 $\mu$ m. Images representative of  $n=9$ . **(B, C)** Single-cell **(B)** and bulk RNA-seq **(C)** data downloaded from <https://immunogenomics.io/ampra/><sup>9</sup> showing the expression of AXL in B cells (SC-B1 to B4), T cells (SC-T1 to T4), fibroblasts (SC-F1 to F4), and monocytes (SC-M1 to M4) and in leukocyte-rich, leukocyte-poor and osteoarthritis synovial tissue.



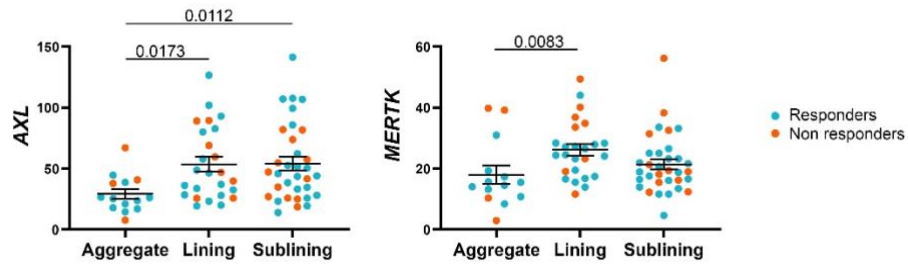
**Supplementary Figure 3** sMerTK and sGas6 expression in the synovial fluid from RA patients according to synovitis score and ESR.

(A, C) Levels of soluble MerTK (sMerTK) and soluble Gas6 (sGas6) in ng/mL assessed by ELISA in the synovial fluid of RA patients (n=18) divided according to synovitis score (low [0-4], high [5-9]). Data are represented as mean  $\pm$  SEM. p-values (not significant) were calculated using the two-tailed Mann Whitney (B, D) Correlation between sMerTK (B) or sGas6 (D) synovial fluid levels and the erythrocyte sedimentation rate (ESR) of RA patients (n=18). p-values (n. s.) calculated according to the two-tailed Spearman correlation test.



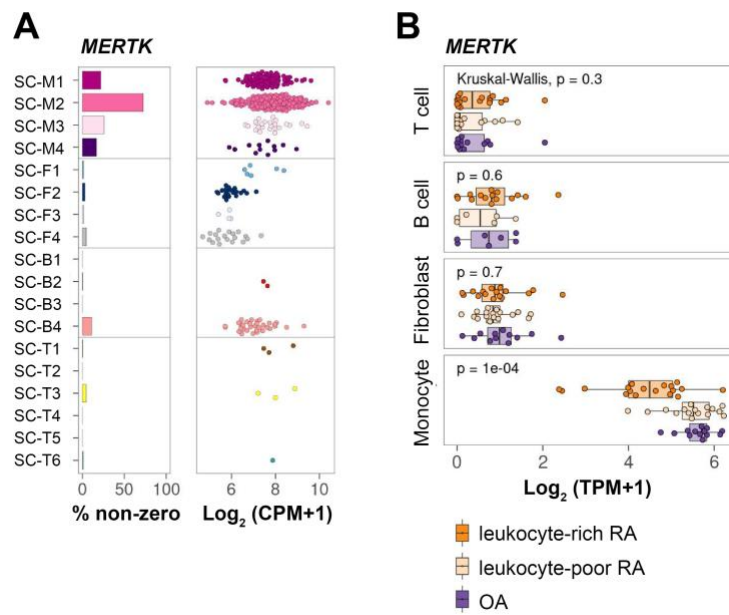
**Supplementary Figure 4 *In vitro* conditioning experiments.**

(A) Schematic representation of the primary synovial fibroblasts (FLS) and macrophages conditioning experiment. Illustrations were created using Servier medical art [smart.servier.com] under Creative Commons Attribution 3.0 Unported License. (B) Expression of Gas6 (pg/mL) in the supernatant of primary fibroblasts-like synoviocytes (FLS) conditioned with supernatant from M1-polarized THP1 (SN M1) or M2-polarized THP1 (SN M2), or in the respective medium used to conditioned the cells (SN CT) (left panel), or in the supernatant of THP1-derived macrophages conditioned with supernatant from unstimulated FLS (SN FLS) or LPS-stimulated FLS (SN FLS<sup>infl.</sup>), or in the respective medium used to conditioned the cells (SN CT) (right panel). Data are represented as mean  $\pm$  SEM. p-values (n. s.) were calculated using the unpaired two-tailed t-test. Experiments were performed on n=3 distinct patient-derived FLS.



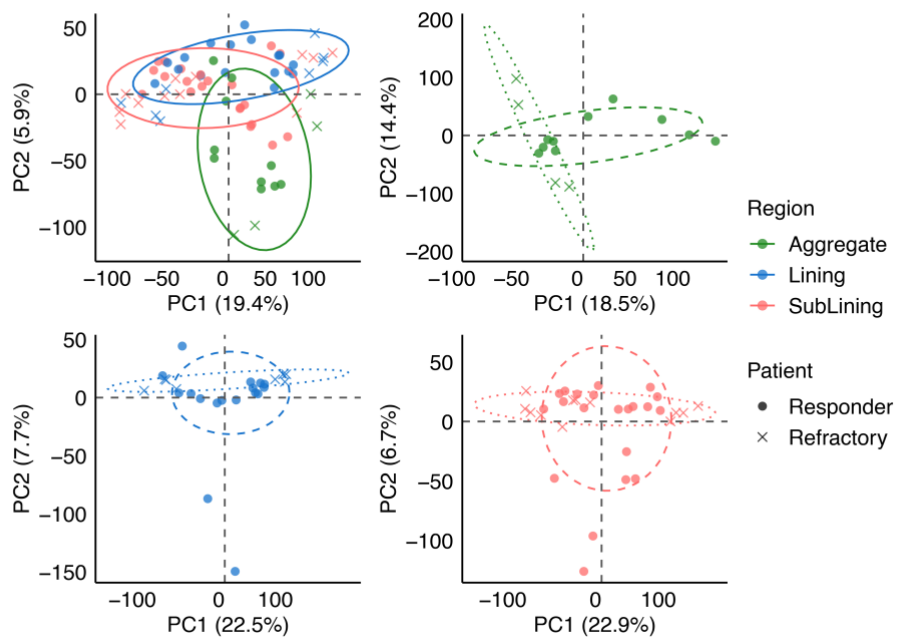
**Supplementary Figure 5 *AXL* and *MERTK* RNA expression varies in distinct synovial regions.**

*AXL* and *MERTK* RNA are differentially expressed in distinct areas of the synovium as quantified by Digital Spatial Profiling (DSP). Both *AXL* and *MERTK* are significantly upregulated in lining and sublining compared to lymphocytic aggregates. Data are represented as mean  $\pm$  SEM. p-values were calculated using the Kruskal–Wallis test with Dunn’s post-test. N=14 aggregates, including 4 non-responders, 10 responders; n= 25 lining, including 8 non-responders and 17 responders; n=33 sublining, including 12 non-responders and 21 responders.



**Supplementary Figure 6** *MERTK* expression in scRNA-seq and bulk RNA-seq public databases.

Single-cell and bulk RNA-seq data downloaded from <https://immunogenomics.io/ampra/><sup>9</sup> showing the expression of *MERTK* in B cells (SC-B1 to B4), T cells (SC-T1 to T4), fibroblasts (SC-F1 to F4), and monocytes (SC-M1 to M4), and in leukocyte-rich, leukocyte-poor and osteoarthritis synovial tissue.



**Supplementary Figure 7 Clustering of the regions of interest in synovial biopsies from rheumatoid arthritis patients.**

Principal component analysis (PCA) of the digital spatial profiling (DSP) of 72 regions of interest (aggregate, lining and sublining) in synovial biopsies from rheumatoid arthritis patients (4 refractory and 8 responders to rituximab or tocilizumab).



**RNA-Seq Cohort (n = 87)**

| <b>Demographics</b>                        |                    |                 |
|--------------------------------------------|--------------------|-----------------|
| Female, % (n)                              |                    | 73.6% (64)      |
| Age, years (mean $\pm$ SD)                 |                    | 53.6 $\pm$ 16.7 |
| Disease duration, months (mean $\pm$ SD)   |                    | 5.9 $\pm$ 3.2   |
| <b>Biochemical and clinical parameters</b> |                    |                 |
| ESR, mm/hr (mean $\pm$ SD)                 |                    | 44.6 $\pm$ 29.4 |
| CRP, mg/l (mean $\pm$ SD)                  |                    | 22.8 $\pm$ 32.9 |
| RF+, % (n)                                 |                    | 71.3% (62)      |
| Anti-CCP+, % (n)                           |                    | 73.6 % (64)     |
| RF and/or anti-CCP+, % (n)                 |                    | 78.2% (68)      |
| TJ/28 joints (mean $\pm$ SD)               |                    | 12.1 $\pm$ 7.6  |
| SJ/28 joints (mean $\pm$ SD)               |                    | 7.9 $\pm$ 5.7   |
| VAS GH patient (mean $\pm$ SD)             |                    | 65 $\pm$ 24     |
| DAS28 (mean $\pm$ SD)                      |                    | 5.9 $\pm$ 1.3   |
| <b>Synovial biopsy site</b>                |                    |                 |
| Biopsied joint                             | MCP/PIP/MTP, % (n) | 13.8% (12)      |
|                                            | Wrist, % (n)       | 63.2% (55)      |
|                                            | Knee, % (n)        | 23% (20)        |
| <b>Histology</b>                           |                    |                 |
| Synovitis score <sup>1</sup>               |                    | 4.7 $\pm$ 2.3   |

**Supplementary Table 1** Baseline clinical characteristics of the patients included in the Pathobiology of Early Arthritis Cohort (PEAC) for RNA-seq analysis (n=87).

n, number; SD, standard deviation; ESR, erythrocyte sedimentation rate; CRP, C-Reactive Protein; RF, rheumatoid factor; CCP, cyclic citrullinated peptide; TJC/28, tender joints count (0-28); SJC/28, swollen joints count/28; VAS GH, Visual Analogue Scale General Health (0-100); DAS28, Disease Activity Score 28; MCP, metacarpophalangeal joints; PIP, proximal interphalangeal joints; MTP, metatarsophalangeal joints.

<sup>1</sup> 10 synovial tissue samples were classified as ungraded. Synovitis score was available for 77 patients.

**Synovial Fluid Cohort (n = 18)**

| <b>Demographics</b>                         |                 |
|---------------------------------------------|-----------------|
| Female, % (n)                               | 72.2 % (13)     |
| Age, years (mean $\pm$ SD)                  | 52.3 $\pm$ 15.6 |
| Disease duration, months (mean $\pm$ SD)    | 4.9 $\pm$ 2.1   |
| <b>Biochemical and clinical parameters</b>  |                 |
| ESR, mm/hr (mean $\pm$ SD)                  | 41.2 $\pm$ 29.7 |
| CRP, mg/l (mean $\pm$ SD)                   | 23.6 $\pm$ 21.5 |
| RF+, % (n) <sup>1</sup>                     | 86.7 % (13)     |
| Anti-CCP+, % (n) <sup>1</sup>               | 80 % (12)       |
| RF and/or anti-CCP+, % (n) <sup>1</sup>     | 86.7 % (13)     |
| TJ/28 joints (mean $\pm$ SD) <sup>2</sup>   | 9.3 $\pm$ 6.3   |
| SJ/28 joints (mean $\pm$ SD) <sup>2</sup>   | 6.4 $\pm$ 5.3   |
| VAS GH patient (mean $\pm$ SD) <sup>2</sup> | 76.3 $\pm$ 17.5 |
| DAS28 (mean $\pm$ SD) <sup>2</sup>          | 5.6 $\pm$ 1     |
| <b>Histology</b>                            |                 |
| Synovitis score                             | 5.3 $\pm$ 2.5   |

**Supplementary Table 2 Baseline clinical characteristics of the patients included in the Pathobiology of Early Arthritis Cohort (PEAC) for synovial fluid ELISA analysis (n=18).**

n, number; SD, standard deviation; ESR, erythrocyte sedimentation rate; CRP, C-Reactive Protein; RF, rheumatoid factor; CCP, cyclic citrullinated peptide; TJC/28, tender joints count (0-28); SJC/28, swollen joints count/28; VAS GH, Visual Analogue Scale General Health (0-100); DAS28, Disease Activity Score 28; MCP, metacarpophalangeal joints; PIP, proximal interphalangeal joints; MTP, metatarsophalangeal joints.

<sup>1</sup> Anti-CCP and RF status available for 15/18 patients.

<sup>2</sup> Number of tender and swollen joints, VAS GH patient and DAS28 available for 17/18 patients